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IgM and IgG Antibodies to Hepatitis E Virus (HEV) Detected by an Enzyme Immunoassay Based on an HEV-Specific Artificial Recombinant Mosaic Protein

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To develop an enzyme immunoassay (EIA) for IgM antibody to hepatitis E virus (HEV) (IgM anti-HEV) and IgG antibody to HEV (IgG anti-HEV), a synthetic gene encoding several liner immunodominant antigenic epitopes from HEV structural proteins was assembled as a chimeric recombinant mosaic protein (Mpr) with glutathione S-transferase and used as an immunodiagnostic target. In addition, a neutralization confirmation test was developed using individual synthetic peptides. Among 614 patients with acute hepatitis from 10 geographically distinct outbreaks, IgG anti-HEV was found in 546 (88.9%), with a range of 77–100% depending on the outbreak. Of 130 patients tested for IgM anti-HEV, 126 (96.9%) were positive. Among patients tested within 4 months of onset of jaundice, 37/37 (100%) were IgG anti-HEV positive. For patients from whom sera were collected 1–16 days after onset of jaundice, the geometric mean IgG titer (GMT) was 1:47,000; the GMT increased to 1:70,710 30–40 days after onset of jaundice and decreased to 1:1,778 3–4 months after the onset of jaundice. For patients tested 6–8 months after onset of jaundice, 11/12 (92%) were IgG anti-HEV positive, and the GMT was 1:2,908. IgM anti-HEV was detected in 43/43 (100%) sera collected 1–40 days after onset of jaundice, and the GMT for IgM anti-HEV was 1:10,000 at that time. For sera collected 3–4 and 6–12 months after onset of jaundice, 7/14 (50%) and 5/12 (40%) respectively, were IgM anti-HEV positive. In conclusion, an artificial mosaic protein composed of linear antigenic epitopes from open reading frame 2 (ORF2) and ORF3 of HEV has been successfully applied to the development of a sensitive and specific EIA for the detection of IgG and IgM anti-HEV activity. These assays were used for the verification of HEV infection in outbreak settings and for the diagnosis of HEV infection in sporadic cases.

KEY WORDS: hepatitis E virus, anti-HEV, enzyme immunoassay, HEV-specific mosaic protein, HEV outbreaks

INTRODUCTION

Many large waterborne outbreaks of enterically transmitted non-A, non-B (ET-NANB) hepatitis with high rates of morbidity and mortality have been reported over the last 4 decades [Wong et al., 1980; Balayan et al., 1983; Favorov et al., 1986; Bradley et al., 1987, 1988; Purcell and Ticehurst, 1988; Zuckerman, 1990]. Until recently, a diagnosis of ET-NANB hepatitis was based on serologically excluding other viral hepatitis. Subsequently, 27–32 nm virus-like particles were identified in stools collected from acutely infected individuals by using immune electron microscopy [Bradley et al., 1987]. More recently, immunofluorescent microscopy [Krawczynski and Bradley, 1989] was used to detect hepatitis E virus (HEV)-specific antibody in sera obtained from experimentally infected animals and from patients with hepatitis E.

In 1990, isolation of a partial cDNA clone from the virus responsible for ET-NANB hepatitis was reported [Reyes et al., 1990]. The authors designated the newly identified agent as the HEV. This clone, derived from a Burma HEV isolate, hybridized with cDNA isolates from

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five other outbreaks in Asia, Africa, and Mexico. These findings strongly suggested that a single agent was responsible for the majority of ET-NANB hepatitis infections seen worldwide [Yarbough et al., 1991; Favorov et al., 1992]. The HEV genome has been characterized as a positive strand RNA molecule, composed of three open reading frames (ORF); the non-structural region is located at the 5′-end and the structural genes are located at the 3′-end [Tam et al., 1991; Huang et al., 1992; Tsarev et al., 1992; Aye et al., 1993; Bi et al., 1993]. Recombinant expressed proteins from ORF2 and ORF3 have been used in the development of a specific immunoblot (IB) and an enzyme immunoassay (EIA) for the detection of anti-HEV activity [Goldsmith et al., 1992; Lok et al., 1992; Bryan et al., 1994]. Studies regarding immunoreactivity of synthetic peptides prepared from ORF2 and ORF3 have been published elsewhere [Cousaget et al., 1993; Kaur et al., 1992; Khudyakov et al., 1993, 1994a,b]. A mixture of synthetic peptides conjugated to bovine serum albumin was used to construct an HEV-specific artificial antigenic complex and to develop an EIA for the detection of anti-HEV activity in sera [Favorov et al., 1994]. Based on the immunoreactivity and specificity of various synthetic peptides, a synthetic gene encoding an artificial polypeptide composed of short antigenic epitopes was constructed from short oligodeoxyribonucleotides using the polymerase chain reaction (PCR) [Khudyakov et al., 1994b]. The protein was expressed in *Escherichia coli* as a chimera with glutathione S-transferase (GST). Because the protein encoded by this gene contains a mosaic of linear antigenic epitopes from ORF2 and epitopes from both the Burmese and Mexican strains from ORF3, this artificial protein was called a mosaic protein (Mpr).

In this paper we report the development of EIAs to detect IgG and IgM anti-HEV using the HEV-specific Mpr as an immunologic target, and the application of these assays to epidemiologic and clinical studies.

MATERIALS AND METHODS

Specimens

Acute-phase (1–23 days after onset of jaundice), unpolled sera were obtained from 614 patients with clinical hepatitis aged 1–67 years who were involved in various outbreaks of ET-NANB hepatitis occurring in 10 different countries between 1982 and 1992; Nepal in 1982 (N = 30), Turkmenistan in 1985 (N = 133), Somalia in 1986 (N = 12), Usbekistan in 1986 (N = 89), Mexico in 1987 (N = 55), Kirgizstan in 1988 (N = 80), Tajikistan in 1990 (N = 63), Kenya in 1991 (N = 119), Iran in 1991 (N = 23), and Angola in 1992 (N = 10). Convalescent-phase sera were also obtained from 69 patients 1–12 months following hospital discharge: Iran (N = 24), Tajikistan (N = 14), and Turkmenistan (N = 31). In addition, sera from the following groups of patients were used as controls: patients with acute hepatitis A, B, C, or D (N = 93); patients with chronic hepatitis C or D virus (N = 25), patients infected with cytomegalovirus, Epstein-Barr virus, human immunodeficiency virus-1, rubella virus (N = 11), or *Toxoplasma gondii;* patients with high levels of C-reactive protein (N = 2); and healthy individuals without a history of jaundice residing in non-HEV endemic regions (United States and Ukraine) (N = 543). All sera collected from healthy individuals were previously tested by IB [Favorov et al., 1992] and peptide EIA [Favorov et al., 1994] and found to be negative for anti-HEV, while sera obtained from jaundiced patients tested positive by both assays.

Buffers

The specimen diluent buffer for IgG detections (SD-G) was composed of 0.01 M phosphate buffered saline (PBS), pH 7.2–7.4, containing 10% normal goat serum, 1% bovine serum albumin, 0.1 mg/ml purified GST, 0.1% Tween-20, and 0.001% sodium azide. The specimen diluent buffer for IgM detection (SD-M) included 7.5 mg/ml of protein A (Boehringer Mannheim, Indianapolis, IN) in addition to SD-G. The conjugate buffer diluent for anti-human IgG and anti-human IgM conjugated to horseradish peroxidase was the same as SD-G without sodium azide.

Mpr Purification

The GST-Mpr fusion protein was purified by affinity chromatography using Glutathione Sepharose 4B (Pharmacia Biotech, Piscataway, NJ) [Smith and Johnson, 1988]. While fraction 2 (0.5 ml) contained the maximum protein concentration (1.2 mg/ml), fractions 3–8 contained the maximum HEV-antigen (HEVAg) activity as determined by EIA and IB. Fractions 3–8 were pooled and used for adsorption to microtiter wells for the detection of anti-HEV in sera by EIA.

Standard Negative and Positive Controls

Five sera negative for anti-HEV by IB [Favorov et al., 1992] and by a peptide EIA [Favorov et al., 1994] that were obtained from healthy donors residing in a non-HEV endemic region were pooled and used as a standard negative control (SNC). For the standard positive control (SPC) a pool of 17 sera was made. These sera were collected from jaundiced patients involved in ET-NANB hepatitis outbreaks in Central Asia [Favorov et al., 1986] and Mexico [Velazquez et al., 1990], and were repeatedly positive by IB and by peptide EIA.

IB Analysis

The purity of the recombinant fusion Mpr (45 kDa) was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Separated proteins were transblotted to BAS 83 nitrocellulose (Schleicher & Schuell, Keene, NH) using a TE70 SemiPhor (Hoeffer, San Francisco, CA).

Removal of GST Carrier by Protease Cleavage

The expressed fusion Mpr contains a site-specific protease cleavage site [Chang, 1985] between the GST protein and the HEV-specific Mpr. After the fusion polypeptide was bound to glutathione-Sepharose 4B beads [Smith and Johnson, 1988], the cleavage of the Mpr from

GST was accomplished by incubation of the column with thrombin (Boehringer Mannheim).

EIA for IgG Anti-HEV Detection

Immunlon II EIA wells (Dynatech Laboratories, Inc., Chantilly, VA) were adsorbed with 105 μl of PBS containing 15 ng of GST-Mpr and incubated overnight at room temperature. After adsorption, each well was washed 5 times with deionized water containing 0.5% Tween-20. Each specimen was diluted 1:10 in SD-G in *naked wells*. Eighty microliters of SD-G was added to each sensitized well and 20 μl of the diluted specimen was added so that the final dilution factor was 1:50. Following an incubation period of 1 hr at 37°C, each well was washed 5 times. Affinity purified goat anti-human IgG, F(ab')2 fragment, conjugated to horseradish peroxidase (Boehringer Mannheim) was diluted 1:30,000 in conjugate buffer and 100 μl was added to each well. The wells were incubated for 1 hr at 37°C and washed 7 times, followed by the addition of 100 μl of substrate solution (o-phenylene diamine and H_2O_2). After incubation for 30 min at room temperature in the dark, the enzyme reaction was stopped with 50 μl of 1 N H_2SO_4. The wells were read in an EIA reader set at 493 nm.

The cutoff value for an initially reactive specimen was statistically derived based on a frequency distribution of optical density (OD) values obtained from 543 healthy persons without a history of jaundice who lived in non-HEV endemic regions and from 90 patients with jaundice who were involved in outbreaks of ET-NANB. For each assay run, the SNC was tested 3 times and the SPC was tested 5 times. The cutoff for each run was equal to an OD value of 0.100 or 3.1 times the mean of the SNC assays. This approximates a cutoff calculation of the mean OD value of the SNC assays + 3.2 standard deviation (SD) of the healthy population sample. In each plate, the OD value of the SPC diluted 1:500 had to be ≥0.35 and the OD value of the SNC had to be 0.01–0.07 for the test to be considered valid.

Neutralization EIA

Neutralization of anti-HEV was achieved by adding unconjugated synthetic peptides to the diluted specimen, as previously described [Khudyakov et al., 1993, 1994a,b; Favorov et al., 1994]. The optimal concentrations of each unconjugated synthetic peptide were as follows: 0.7 mg/ml for peptides 28, 29, and 23; 0.5 mg/ml for peptides 5, 12, and 22; and 0.3 mg/ml for peptide 40. Specimens that initially tested positive by the screening test for anti-HEV were retested simultaneously with and without neutralization at an initial specimen dilution of 1:50. The first row contained sera diluted in SD-G and the second row contained sera diluted in SD-G with unconjugated peptides, so that the same sera were located in two wells in a vertical position. Initially reactive sera that yielded OD values greater than the cutoff at a 1:50 dilution that were also not neutralized were retested at a higher dilution. Sera were considered to be confirmed positive for anti-HEV when the OD value obtained without synthetic peptides was reduced by at least 50% after neutralization with peptides.

EIA for IgM Anti-HEV Detection

Two different approaches were used for the detection of IgM anti-HEV. The first utilized anti-human IgM (μ-chain specific) adsorbed to microtiter wells to capture IgM from sera, followed by the addition of GST-Mpr and human anti-HEV conjugated to horseradish peroxidase. Because of an unexpectedly low efficiency of interaction between captured IgM and the Mpr (data not shown), this assay format was abandoned.

The second approach to detect IgM anti-HEV was based on two modifications of the EIA for IgG anti-HEV detection: 1) addition of various reagents to reduce or eliminate IgG anti-HEV activity, and 2) replacement of the anti-human IgG conjugate with anti-human IgM (μ-chain specific) conjugate. Several reagents were tested to reduce IgG anti-HEV activity, including proteins A and G and anti-human IgG (Boehringer Mannheim). The most effective reagent in this format was protein A at a concentration of 1.5 μg/ml in SD-G (SD-M buffer).

Immunlon II EIA wells (Dynatech Laboratories, Inc.) were adsorbed with 105 μl of PBS containing 15 ng of GST-Mpr and incubated overnight at room temperature. After adsorption, each well was washed 5 times with deionized water containing 0.5% Tween-20. Each specimen was diluted 1:20 in SD-M in naked wells. Ninety-five microliters of SD-M was added to each sensitized well and 5 μl of the diluted specimen was added so that the final dilution factor was 1:400. Following overnight incubation at room temperature, each well was washed 5 times. Affinity purified goat anti-human IgM conjugated to horseradish peroxidase (Boehringer Mannheim) was diluted 1:15,000 in conjugate buffer and 100 μl was added to each well. The wells were incubated for 1 hr at 37°C and washed 7 times, followed by the addition of 100 μl of substrate solution. After incubation for 30 min at room temperature in the dark, the enzyme reaction was stopped with 50 μl of 1 N H_2SO_4. The wells were read in an EIA reader set at an OD of 493 nm.

The cutoff value for an initially reactive specimen was statistically derived based on a frequency distribution of OD values in 141 sera obtained from healthy donors from non-endemic regions and from 68 sera obtained 1–15 days after onset of jaundice from patients involved in outbreaks of ET-NANB hepatitis. In each plate the OD value of SPC diluted 1:500 had to be ≥0.5 for the test to be considered valid. An SNC was used as described above by IgG detection.

Fractionation of Sera

Anti-HEV negative (0.5 ml) and anti-HEV positive specimens were chromatographically fractionated through a fast protein liquid chromatographic (FPLC) Superose-12 column (Pharmacia-Biotech, Piscataway, NJ) equilibrated in 5% acetic acid at a flow rate of 0.2 ml/min. Each fraction was lyophilized and subsequently reconstituted in H_2O and tested by EIA for IgG and IgM anti-HEV.

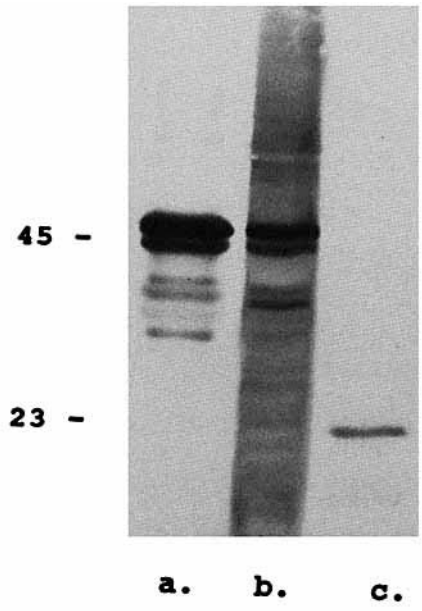

Fig. 1. IB analysis of the HEV-specific artificial Mpr using anti-HEV positive serum. **a:** Purified fusion GST-Mpr. **b:** Crude lysate containing fusion GST-Mpr. **c:** Cleaved Mpr.

Titration Study

Serum samples were serially diluted 10-fold in SD-G or SD-M, from 1:100 to 1:1,000,000. Each dilution was tested for IgG and IgM anti-HEV by EIA.

Serologic Testing

Sera specimens were tested for markers of HAV, HBV, HDV, and HCV infection using commercially available kits (Abbott Laboratories, North Chicago, IL; NPO Diagnostic Systems, Nizhny Novgorod, Russia).

Statistical Analysis

Statistical analyses were done using the Pearson χ^2 test, the Mantel-Haenszel χ^2 test, and the χ^2 test for trend, as appropriate. Statistical significance was determined by calculating *P* values with Yates correction factor and exact 95% confidence intervals.

RESULTS

Mpr

Following affinity chromatography purification, the Mpr was analyzed by SDS-PAGE and IB. The Mpr represented approximately 70–80% of the total protein after purification, and almost 100% after thrombin cleavage (data not shown). However, the yield of cleaved Mpr was only about 10%. Figure 1 represents specific immunoreactivity of the Mpr as measured by IB using sera obtained from patients with acute hepatitis E.

Anti-HEV Activity in Fractionated Sera

Sera obtained from a patient with acute hepatitis E 5 days after onset of jaundice and from a healthy donor (control) were fractionated by FPLC. In the specimen from the hepatitis E patient three peaks of activity were identified (Fig. 2). IgM anti-HEV was found in fraction 25, while IgG anti-HEV was resolved into two peaks (fractions 36 and 41). Anti-HEV was not detected in the control specimen.

Frequency Distribution of IgG Anti-HEV Activity

Using SNC and SPC, the optimal concentration of Mpr adsorbed to microtiter wells was 0.15 μg/ml, which yielded a positive to negative (P/N) value of 23. Sera from 543 persons without a history of clinical hepatitis who resided in non-HEV endemic regions and sera from 90 patients with acute hepatitis and jaundice who were involved in ET-NANB outbreaks between 1985 and 1987 in the Central Asian Republics of the former Soviet Union and in Mexico were used to derive an EIA frequency distribution of OD values for positive and negative specimens. The range of OD values among the 543 specimens collected from healthy individuals was 0.001–0.246. Of the 543, 490 (90.2%) yielded OD values <0.1. The arithmetic mean of OD values was 0.048 (±0.043 SD). Thus, 3.2 SD above the mean corresponded to an OD cutoff value of 0.186. Only two (0.34%) specimens exceeded the cutoff (0.196 and 0.246), and anti-HEV activity in these two specimens was confirmed by the neutralization assay. All 90 specimens from patients with jaundice during the ET-NANB outbreaks had OD values >0.3 (Fig. 3).

EIA Based on Cleaved Mpr

Cleaved Mpr was compared to the fusion protein as the target protein. Among 47 randomly selected anti-HEV positive and negative sera, 16 were positive for both antigens. Because of the simplicity of obtaining purified GST-Mpr and the absence of any apparent advantage of using the cleaved Mpr for EIA, the fusion protein was used for all subsequent experiments.

Neutralization EIA

For confirmation of anti-HEV positive results, a neutralization test was developed. Figure 4 presents the results of the test using 68 randomly selected sera that tested positive by Mpr EIA with OD values between 0.2 and 2.5: 25 (37%) had OD values between 0.2 and 0.5; 22 (32%) between 0.51 and 1.0; 13 (19%) between 1.01 and 1.5; and 8 (12%) >1.5. Overall, 58 (97%) sera with OD values <1.5 were confirmed as positive, with the degree of neutralization ranging from 52 to 99%. The mean percent of neutralization ranged from 65 to 87% depending on initial OD values. Two specimens that did not neutralize had OD values of 0.210 and 0.223 and were classified as non-specific reactive samples. Sera that yielded OD values >2.5 did not neutralize when tested at a 1:50 dilution; however, when diluted to 1:500, incubated with the mixture of synthetic peptides, and retested, both samples were neutralized >50%.

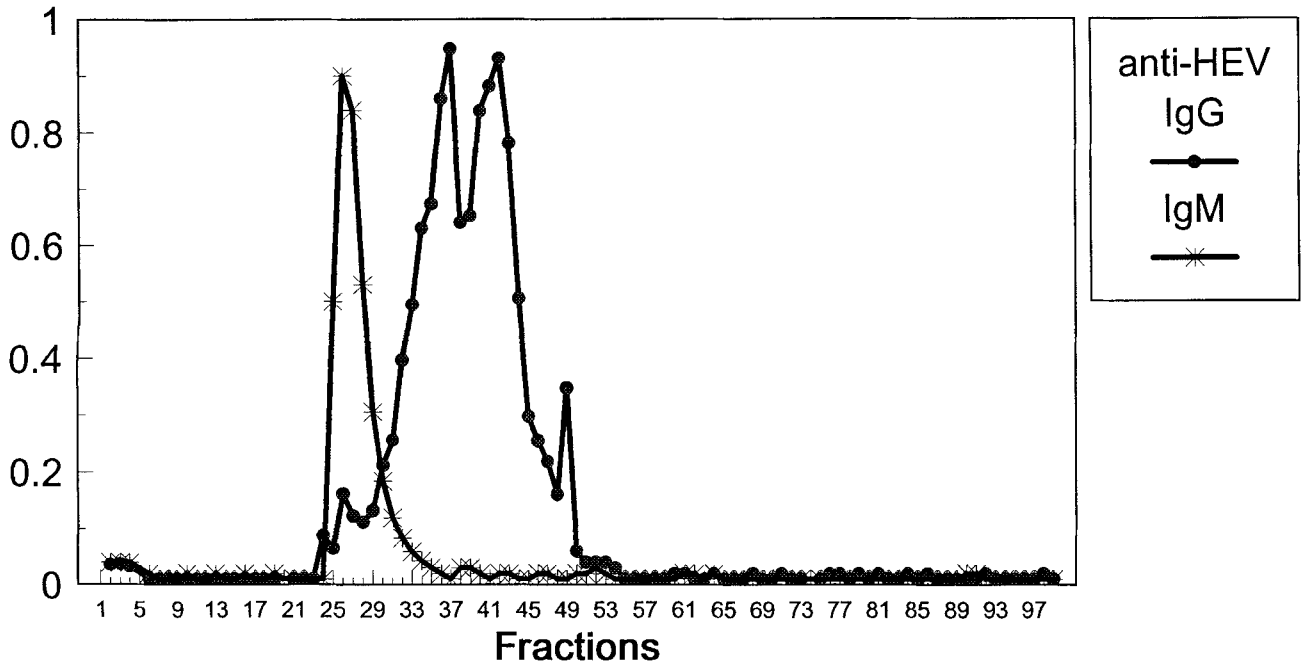

Fig. 2. Subclasses of anti-HEV fractionated from an acute-phase specimen measured by Mpr EIA.

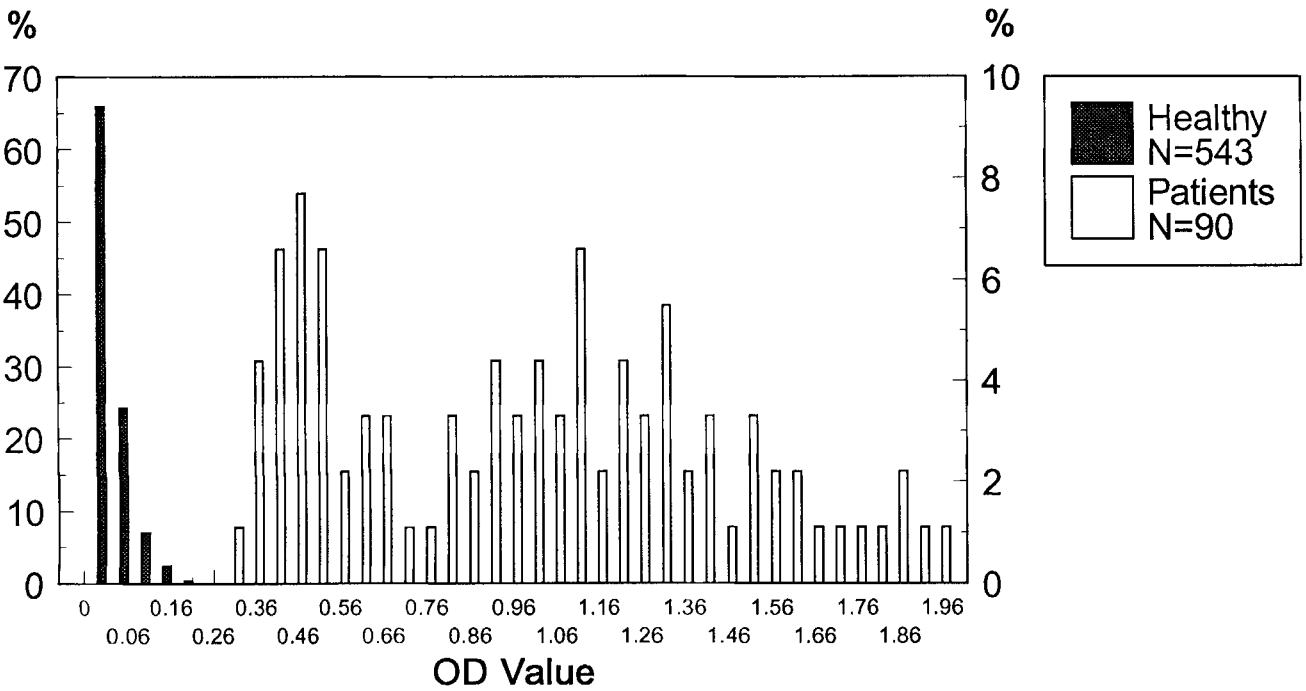

Fig. 3. Frequency distribution of OD value for sera of healthy individuals and HEV-infected patients.

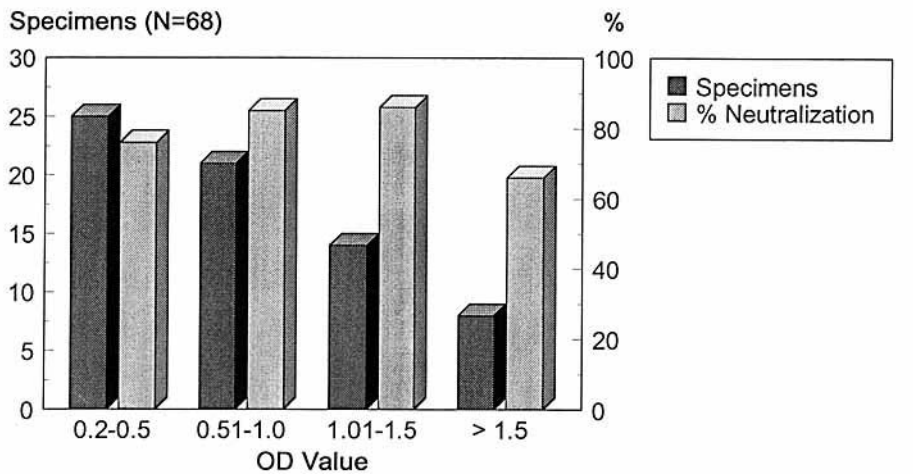

Fig. 4. IgG anti-HEV confirmation test.

TABLE I. Anti-HEV Activity by Mpr-Base EIA Among Patients Involved in ET-NANB Outbreaks

Regions	Year	No. of patients	Anti-HEV IgG (%)	Anti-HEV IgM (%)
Nepal	1982	30	30 (100)	30 (100)*
Turkmenistan	1985	133	121 (90.9)	
Somalia	1986	12	12 (100)	12 (100)
Usbekistan	1986	89[a]	72 (81)	
Mexico	1987	55	54 (98)	53 (96)
Kirgizstan	1988	80	70 (88)	
Tajikistan	1990	63	63 (100)	
Iran	1991	23	22 (96)	21 (91)*
Kenya	1991	119[a]	92 (77.3)	
Angola	1992	10	10 (100)	10 (100)
Total		614	546 (88.9)	130/126 (96.9)

[a]Specimens collected from reported cases.
*$P > 0.1$.

Anti-HEV Activity Among Controls

None of the 131 acute-phase sera positive for markers of HBV, HDV, HCV, and other diseases was positive for IgG.

Detection of IgG Anti-HEV in Epidemiologically Defined Populations

The Mpr EIA was used to detect anti-HEV (Table I) in sera collected in various epidemiologic settings. Among 614 patients with jaundice involved in 10 geographically distinct outbreaks of ET-NANB hepatitis in Angola, the Central Asian region of the former Soviet Union, Iran, Kenya, Mexico, and Nepal, anti-HEV was detected in 546 (88.9%), ranging from 77 to 100% depending on the outbreak.

Frequency Distribution of IgM Anti-HEV Reactivity

A frequency distribution of OD values for IgM anti-HEV activity was generated using 141 sera collected from healthy donors residing in non-endemic regions and 68 sera obtained 1–15 days after onset of jaundice from patients involved in ET-NANB hepatitis outbreaks in Angola, Iran, Nepal, Mexico, and Somalia. The arithmetic mean of OD values of the healthy donors was 0.062 ± 0.072 SD. Based on EIA results, three zones were defined: positive (>4.5 SD above the mean of negative controls), negative (<3.4 SD above the mean of negative controls), and intermediate (3.4–4.5 SD above the mean of negative controls). Of the 68 sera patients with jaundice during ET-NANB hepatitis outbreaks, 63 (93%) had OD values in the positive zone and 5 (7%) had OD values in the intermediate zone; all sera from healthy donors had OD values in the negative zone.

Detection of IgM Anti-HEV in Epidemiologically Defined Populations

Sera from 130 patients with acute HEV infection involved in 5 geographically distinct outbreaks were tested for IgM anti-HEV (Table I). IgM anti-HEV was found in 126 (96.9%) patients. From these outbreaks, *the pro-*

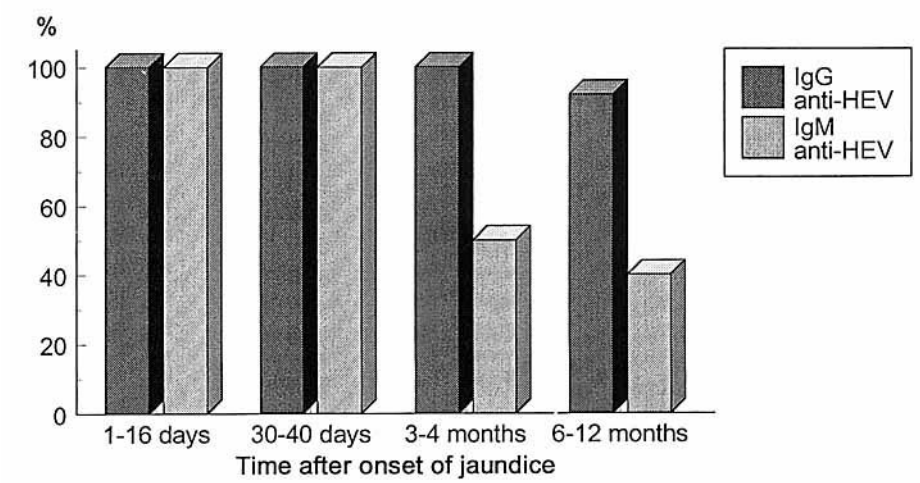

Fig. 5. Patients (N = 69) positive for IgM and IgG anti-HEV with the Mpr EIA, by time after onset of jaundice.

portion of patients with IgM anti-HEV ranged from 91 to 100%.

Persistence of Anti-HEV After Acute Infection

Sixty-nine follow-up specimens collected from patients involved in various HEV outbreaks were analyzed by the Mpr EIA to determine the persistence of IgG and IgM anti-HEV during the natural course of infection (Fig. 5). IgG anti-HEV was found among 37/37 (100%) specimens collected within 4 months after onset of jaundice and in 11/12 (92%) collected 6–12 months after onset ($P > 0.2$). The geometric mean titer (GMT) for IgG anti-HEV was 1:47,000 in sera collected 1–16 days after onset of jaundice; 1:70,710 30–40 days after; 1:1,778 3–4 months after; and 1:2,908 6–12 months after ($P > 0.1$) (Fig. 6). IgM anti-HEV was detected in 43/43 (100%) sera collected 1–40 days after onset of jaundice; 7/14 (50%) 3–4 months after; and 5/12 (40%) 6–12 months after. The GMT for IgM anti-HEV was 1:11,406 in sera collected 1–16 days after onset of jaundice; 1:10,000 30–40 days after; 1:19 3–4 months after; and 1:16 6–12 months after.

DISCUSSION

Several immunodominant regions in the HEV proteins encoded by ORF2 and ORF3 have been identified [Khudyakov et al., 1993, 1994a,b; Favorov et al., 1994]. Three regions of strong antigenic activity have been found in the ORF2 protein, including a region at amino acid (aa) position 394–470, which contains several antigenic epitopes. In the ORF3-encoded protein, the C-terminal region at aa position 91–123 has demonstrated strong antigenic immunoreactivity [Cousaget et al., 1993; Bryan et al., 1994]. Because of nucleotide variations found in the ORF3 of the Burmese and Mexican HEV strains, the Mpr included the C-terminal region from both. The synthetic gene encoding this Mpr was assembled by PCR and expressed as previously described [Khudyakov et al., 1994a,b].

The results of Mpr EIA suggest that the IgG and IgM anti-HEV assays are specific for each class of antibody. In FPLC fractionated sera obtained 5 days after the onset of jaundice from a patient with acute hepatitis E, at least three peaks of anti-HEV activity were found (Fig. 2). Activity in fraction 41 may represent multimers of IgG, although no further experiments were conducted.

The frequency distribution of anti-HEV among sera obtained from healthy individuals residing in a non-endemic region and among sera from patients with acute hepatitis E residing in an endemic region demonstrated the high specificity of the Mpr EIA by a clear zone separating negative and positive parts of the curve. Despite this observation and considering that anti-HEV titers significantly decline over time, a confirmation test was developed.

Neutralization of anti-HEV by individual HEV-specific synthetic peptides was used as a confirmatory test for IgG detection. The use of the neutralization test significantly lowered the false-positive rate, especially in sera that had been improperly stored, had undergone several freeze thaw cycles, or had been microbially contaminated (data not shown). The reagents used for neutralization were different from the material adsorbed to the microtiter wells so that false positive reactions would not be incorrectly neutralized.

The EIA test described here recognized anti-HEV among patients from a variety of outbreaks. The percentage of IgG anti-HEV positive patients involved in the 10 outbreaks ranged from 77 to 100%. The relatively low rate of anti-HEV from outbreaks in Kenya (77%) and Usbekistan (81%) may be due to the way in which acute cases were identified. Samples from these two outbreaks were collected from reported jaundice patients, rather than from hospitalized patients as in the other eight outbreaks.

Tests for IgM anti-HEV are essential for the verification of a diagnosis of acute infection. A direct μ-chain

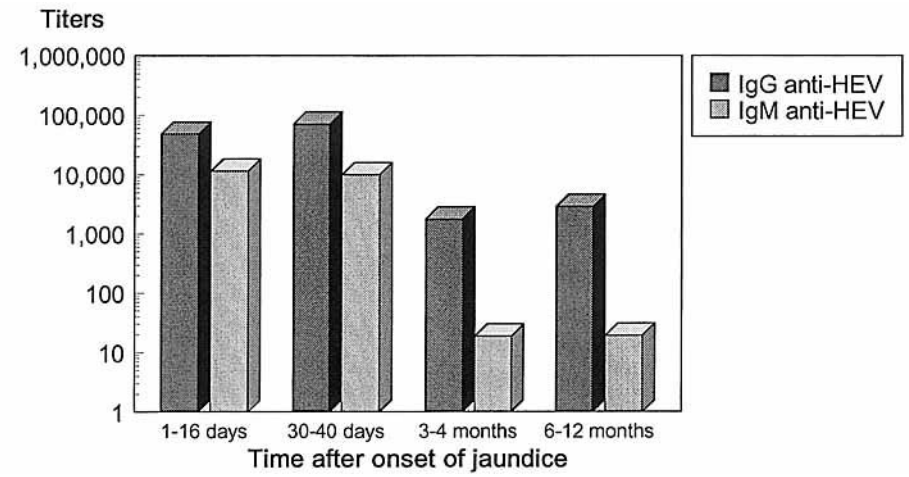

Fig. 6. Geometric mean of anti-HEV titers as measured by Mpr EIA.

capture technique for the detection of IgM anti-HEV was not successful. One possible explanation may be a relatively low affinity of IgM anti-HEV to the existing version of recombinant Mpr. The second approach to detect IgM anti-HEV was based on the addition of protein A to the test specimen to reduce or eliminate competing IgG anti-HEV activity, and the substitution of anti-human IgG with anti-human IgM conjugated to horseradish peroxidase. According to published reports [Goldsmith et al., 1992; Dawson et al., 1993; Mushahwar et al., 1993; Bryan et al., 1994], between 55 and 95% of anti-HEV positive specimens obtained from patients within 1 month of onset of jaundice had IgM anti-HEV as detected by similar approaches. This percentage may be increased by blocking the competing IgG activity for the target Mpr. Unlike IgG anti-HEV, the cutoff for IgM detection is usually based upon seroconversion panels. In the absence of available seroconversion panels, a frequency distribution was used. Thus, the duration of IgM anti-HEV detection in a population of patients with acute infection and the specificity of the assay in persons with remote infection are not known.

IgG anti-HEV was found among all patients from day 1 to 4 months after onset of jaundice, and in 92% 6–12 months after. IgM anti-HEV was detected in all sera collected between 1 and 40 days after onset of jaundice; however, a lower percentage (50%) was detected in patients between 3 and 4 months after onset. These data are similar to the results of a previous study obtained by IB analysis and EIA analysis [Favorov et al., 1992; Dawson et al., 1993]. As expected, the IgG anti-HEV titer also decreased from about 1:70,000 after 30–40 days of onset of jaundice to about 1:2,000 3–4 months later. The data suggest that anti-HEV titer is also a useful indicator of acute HEV infection, like IgM anti-HEV detection. Similarly, the GMT for IgM anti-HEV declined from 1:11,000 within the first month of the disease to almost undetectable levels 3–4 months after onset of jaundice. Bryan et al. [1994] described a similar distribution of anti-HEV titers during the natural course of the disease using an EIA based on recombinant HEV ORF2 proteins, although the actual titers were significantly lower (i.e., 1:1,000–1:2,500 for IgM and 1:1,500–1:5,500 for IgG). The differences in titers may be related to the design of the respective EIAs and the availability of additional epitopes from ORF3 as found in the Mpr.

An artificial Mpr composed to linear antigenic epitopes from ORF2 and ORF3 has been applied successfully to the development of a sensitive and specific EIA for the detection of IgG and IgM anti-HEV. These assays were used for the verification of HEV infection in outbreak settings. The IgG neutralization assay was also shown to be of value in avoiding false-positive results. Additional research is in progress to assess whether the Mpr can be improved by adding or duplicating certain linear antigenic epitopes, or by adding conformational epitopes if they are found to be diagnostically relevant. Research is also in progress to describe the distribution of HEV infections in various regions of the world and to study the pathogenesis of various forms of the disease.

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